PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing (day/month/year)	27 November 2000 (27.11.00)
Applicant's or agent's file reference	IMPORTANT NOTIFICATION
PA/KIST99346	

International application No.	International filing date (day/month/year)	Priority Date (day/month/year)
PCT/ KR 99/00414	30 July 1999 (30.07.99)	31 July 1998 (31.07.98)

Applicant
Korea Institute of Science and Technology et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the eleceted Offices.

3. Where required by any of the elected Offices, the Interational Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the eleceted Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/AT	Authorized officer
Austrian Patent Office Kohlmarkt 8-10 A-1014 Vienna Facsimile No. 1/53424/200	Koch Telephone No. +43 / 1 / 53424 - 450

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
PA/KIST 99346		
International application No.	International filing date (*day/month/year*)	Priority Date (*day/month/year*)
PCT/KR 99/00414	30 July 1999 (30.07.99)	31 July 1998 (31.07.98)
International Patent Classification (IPC) or national classification and IPC		
IPC[7]: A61K 9/0107		
Applicant		
KOREA INSTITUTE OF SCIENCE AND TECHNOLOGY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examination Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 10 sheets.

3. This report contains indications relating to the following items:

   - I ☐ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
28 February 2000 (28.02.00)	27 September 2000 (27.09.00)
Name and mailing address of the IPEA/AT Austrian Patent Office Kohlmarkt 8-10 A-1014 Vienna Facsimile No. 1/53424/535	Authorized officer Mosser Telephone No. 1/53424/437

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/KR 99/00414

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages 1-34 , as originally filed
pages _____ , filed with the demand
pages _____, filed with the letter of _____.

☒ the claims:
~~pages~~ _____ , ~~as originally filed~~
~~pages~~ _____ , ~~as amended (together with any statement) under Article 19~~
pages 35-40, 44 , filed with the demand
pages 41-43 filed with the letter of 25 September 2000 (25.09.00).

☒ the drawings:
pages 1/25 – 25/25
pages _____ , as originally filed
pages _____ , ~~filed with the letter of~~ _____ , filed with the demand

☐ the sequence listing part of the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____.

☐ the claims, Nos. _____.

☐ the drawings, sheets/fig _____.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as „originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/KR 99/00414

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1, 2, 5, 6, 9-25, 52-58, 60-72, 75, 76, 79, 80	YES
	Claims		NO
Inventive step (IS)	Claims	1, 2, 5, 6, 9-25, 52-58, 60-72, 75, 76, 79, 80	YES
	Claims		NO
Industrial applicability (IA)	Claims	1, 2, 5, 6, 9-25, 52-58, 60-72, 75, 76, 79, 80	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

With regard to the new claims 58 and 68, dated 25 September 2000 (25.09.00), novelty, inventive step and industrial applicability are recognized for all claims.

Claims;

1. An oil-in-water lipid emulsion for delivering biologically active material selected from the group consisting of DNA, RNA, antisense nucleic acid, ribosome, polynucleotide and oligonucleotide, comprising: 2-30% of non-triglyceride oil; 0.01-20% of one or more cationic lipid transfection agent; and, water to 100%.

2. Solid-lipid nanoparticles for delivering biologically active material selected from the group consisting of DNA, RNA, antisense nucleic acid, ribosome, polynuleotide and oligonucleotide, comprising: 2-30% of fat of triglycerides having 10-18 carbons in each hydrophobic tail or ethyl stearate; 0.01-20% of one or more cationic lipid transfection agent; and, water to 100%.

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5. A method of preparing an oil-in-water lipid emulsion for delivering biologically active material selected from the group consisting of DNA, RNA, antisense nucleic acid, ribosome, polynucleotide and oligonucleotide, comprising: a) a first step of preparing an aqueous phase by mixing 0.01-20% of one or more cationic lipid transfection agent with water and b) a second step of preparing emulsion of said aqueous phase with 2-30% of non-triglyceride oil.

6. A method of preparing solid lipid nanoparticles for delivering biologically active material selected from the group consisting of DNA, RNA, antisense nucleic

acid, ribosome, polynucleotide and oligonucleotide, comprising: a) a first step of preparing an aqueous phase by mixing 0.01-20% of one or more cationic lipid transfection agent with water and b) a second step of mixing said aqueous phase with 2-30% of fat of triglycerides having 10-18 carbons in each hydrophobic tail or ethyl stearate.

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9. The emulsion according to claim 1, further comprisign 0.01-10% of a hydrophilic polymer or hydrophilic polymeric lipid.

10. The emulsion accordign to claim 1, wherein the non-triglycerides is squalene or squalane.

11. The emulsion according to any of claims 1, 9 or 10, further comprising a phospholipid or a non-ionic surfactant.

12. The emulsion according to claim 1, wherein the cationic lipid transfection agent is selected from the group consisting of:

1,2-dimyristoyl-3-trimethylammonium-propane,

1,2-dipalmitoyl-3-trimethylammonium-propane,

1,2-distearoyl-3-trimethylammonium-propane,

1,2-dioleoyl-3-trimethylammonium-propane,

1,2-dimyristoyl-3-dimethylammonium-propane,

1,2-dipalmitoyl-3-dimethylammonium-propane,

1,2-dilauroyl-3-dimethylammonium-propane,

1,2-distearoyl-3-dimethylammonium-propane,

1,2-dipalmitoyl-3-trimethylammonium-propane,

N-[1-(1,2-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride,

1,2-dioleoyl-3-ethylphosphocholine, and other cationic lipids.

13. The emulsion according to any of claims 1, 9, or 10, further comprising glycerol or fusogenic peptides.

14. The emulsion according to claim 13, wherein the fusogenic peptide is polyethylene glycol of MW.500-1000 or HA gp 41.

15. The emulsion according to claim 9, wherein the hydrophilic polymer is selected from the group consisting of polyoxyethylene, polyethyloxazoline and polyethyleneglycol.

16. The emulsion according to claim 11, wherein the phospholipid is selected from the group consisting of phosphatidylcholin, phosphatidylethanolamine, phosphatidylserine, diacetylenic phospholipid and derivative thereof and the non-ionic surface active agent is selected from the group consisting of poloxamer, sorbitan ester, polyoxyethylene-sorbitan fat acid ester and polyoxyethylene ethers.

17. The emulsion according to any of claims, 1, 9, or 10, further comprising 1,2-dioleoyl-sn-3-phosphatidylethanolamine, diolein, fatty alcohol, cholesterol

or bile salt.

18. The solid lipid nanoparticles according to claim 2, further comprising 0.01-10% of a hydrophilic polymer or hydrophilic polymeric lipid.

19. The solid lipid nanoparticles according to claim 2 or 18, further comprising a phospholipid or a non-ionic surfactant.

20. The solid lipid nanoparticle according to claim 2, wherein the cationic lipid transfection agent is is selected from the group consisting of:

1,2-dimyristoyl-3-trimethylammonium-propane,

1,2-dipalmitoyl-3-trimethylammonium-propane,

1,2-distearoyl-3-trimethylammonium-propane,

1,2-dioleoyl-3-trimethylammonium-propane,

1,2-dimyristoyl-3-dimethylammonium-propane,

1,2-dipalmitoyl-3-dimethylammonium-propane,

1,2-dilauroyl-3-dimethylammonium-propane,

1,2-distearoyl-3-dimethylammonium-propane,

1,2-dipalmitoyl-3-trimethylammonium-propane,

N-[1-(1,2-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride,

1,2-dioleoyl-3-ethylphosphocholine, and other cationic lipids.

21. The solid lipid nanoparticles according to claim 2 or 18, further

comprising glycerol or fusogenic peptides.

22. The solid lipid nanoparticles according to claim 21, wherein the fusogenic peptide is polyethylene glycol of MW.500-1000 or HA gp 41.

23. The solid lipid nanoparticles according to claim 18, wherein the hydrophilic polymer is selected from the group consisting of polyoxyethylene, polyethyloxazoline and polyethyleneglycol.

24. The solid lipid nanoparticles accordign to claim 19, wherein the phospholipid is selected from the group consisting of phosphatidylcholin, phosphatidylethanolamine, phosphatidylserine, diacetylenic phospholipid and derivative thereof and the non-ionic surface active agent is selected from the group consisting of poloxamer, sorbitan ester, polyoxyethylene-sorbitan fat acid ester and polyoxyethylene ethers.

25. The solid lipid nanoparticles according to claim 2 or 18, further comprising 1,2-dioleoyl-sn-3-phosphatidylethanolamine, diolein, fatty alcohol, cholesterol or bild salt.

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52. A complex of the emulsion according to any of claims 1, 9 to 17 and a biologically active material selected from the group consisting of DNA, RNA, antisense nucleic acid, ribosome, polynuleotide, oligonucleotide.

53. The complex according to claim 52, further comprising glycolipid, lipopeptide, antibody, ligand for receptors or viral protein to target specific cells or organs.

54. The complex according to claim 52 or 53, further comprising protamine sulfate, histone or cationic polymer.

55. The complex according to claim 54, wherein cationic polymer is polylysine.

56. The complex according to claim 52, further comprising monovalent or multivalent salt.

57. The complex according to claim 53, wherein the cell is selected from the group consisting of white blood cells, fibroblasts, cancer cells, cells infected with virus, epithelial cells, endothelial cells, muscle cells, liver cells, endocrine cells, neural cells, dermal cells, germ cells, oocytes, sperms, hematopoietic cells, fetal cells, M cells, Langerhans islet cells, marcophages, plant cells, animal cells, and immortalized cell lines.

58. The complex according to claim 52, wherein the complex is to be transferred to cells via intravenous, intramuscular, intratracheal, intranasal,

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subcutaneous, parenteral or topical administration or via direct administration to a specific organ.

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60. The complex according to claim 52, further comprising lipophilic or amphiphilic drug in an oil phase, wherein the lipophilic or amphiphlic drug is selected from the group consisting of antivirals, steroidal anti-inflammatory drugs, non-steroidal anti-inflammatory drugs, antibiotics, antifungals, vitamins, hormones, retinoic acid, prostaglandins, prostacyclins, anticancer drugs, antimetabolitic drugs, miotics, cholinergics, adrenergic antagonists, anticonvulsants, antianxiety agents, major tranquilizers, antidepressants, anesthetics, analgesics; anabolic steroids, estrogens, progesterones, glycosaminoglycans, polynucleotides, immunosuppressants and immunostimulants.

61. The complex according to claim 60 wherein the anticancer drug is taxol, paclitaxel or flourourcil.

62. A complex of the solid lipid nanoparticles according to any of claims 2, 12 to 19 with a biologically active material selected from the group consisting of DNA, RNA, antisense nucleic acid, ribosome, polynucleotide and oligonucleotide.

63. The complex according to claim 62, further comprising glycolipid, lipop ptide, antibody, ligand for receptors r viral protein to target specific cells or organs.

64. The complex according to claims 62 or 63, further comprising protamine

sulfate, histone or cationic polymer.

65. The complex according to claim 64, wherein the cationic polymer is polylysine.

66. The complex according to claim 62, further comprising monovalent or multivalent salt.

67. The complex according to claim 63, wherein the cell is selected from th group consisting of white blood cells, fibroblasts, cancer cells, cells infected with virus, epithelial cells, endothelial cells, muscle cells, liver cells, endocrine cells, neural cells, dermal cells, germ cells, oocytes, sperms, hematopoietic cells, fetal cells, M cells, Langerhans islet cells, marcophages, plant cells, animal cells, and immortalized cell lines.

68. The complex according to claim 62, wherein the complex is to be transferred to cells via intravenous, intramuscular, intratracheal, intranasal, subcutaneous, parenteral or topical administration or via direct administration to a specific organ.

69. The complex according to any of claims 62 to 68, further comprising lipophilic or amphiphilic drug in the fat, wherein the lipophilic or amphiphlic drug is selected from the group consisting of antivirals, steroidal anti-inflammatory drugs, non-steroidal anti-inflammatory drugs, antibiotics, antifungals, vitamins, hormones, retinoic acid, prostaglandins, prostacyclins, anticancer drugs, antimetabolitic drugs, miotics, cholinergics, adrenergic antagonists, anticonvulsants, antianxiety agents, major tranquilizers, antidepressants, anesth tics, analg sics, anabolic steroids, strogens, progesterones,